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(54) Title: PEPTIDE LIBRARY AND SCREENING METHOD

(57) Abstract

A random peptide library constructed by transforming host cells with a collection of recombinant vectors that encode a fusion protein comprised of a DNA binding protein and a random peptide and also encode a binding site for the DNA binding protein can be used to screen for novel ligands. The screening method results in the formation of a complex comprising the fusion protein bound to a receptor through the random peptide ligand and to the recombinant DNA vector through the DNA binding protein.

WHAT IS CLAIMED IS:

1. A method of isolating a DNA binding protein comprising:

(a) providing a recombinant DNA vector comprising a coding sequence for a peptide having a specific affinity for a receptor;

(b) inserting a library of oligonucleotides encoding different potential DNA binding proteins into the vector in-frame with the peptide coding sequence to form a library of different vectors encoding different fusion proteins, the fusion proteins differing in the potential DNA binding protein;

(c) transforming host cells with the vectors;

(d) culturing the transformed host cells under conditions suitable for expression of the fusion proteins, whereby, if a fusion protein comprises a potential DNA binding protein with affinity for the vector, the fusion protein binds to the vector to form a complex;

(e) lysing the transformed host cells under conditions such that complexes formed in (d) remain associated;

(f) contacting the complexes with a receptor under conditions conducive to specific binding of the peptide to the receptor;

(g) isolating complexes bound to the receptor, the complexes containing vectors encoding DNA binding proteins.

2. The method of claim 1, further comprising isolating the vectors from the complexes in (g), and repeating (c)-(g).

3. The method of claim 2, further comprising determining the sequence of a DNA binding protein encoded by a vector in (g).

4. The method of claim 3, further comprising:

transforming the vector in (g) into host cells under conditions suitable for expression of the fusion protein

encoded by the vector, whereby the fusion protein binds to the vector to form a complex;

lysing the transformed host cells under conditions such that the complex remains associated;

contacting separate samples of the complex to the receptor and to a receptor lacking affinity for the peptide under conditions conducive to specific binding of the peptide to the receptor;

isolating vector from: (1) complex bound to the receptor and (2) complex bound to the receptor lacking affinity for the peptide;

separately transforming vector obtained from (1) and (2) and calculating an enrichment ratio equal to transformants from (1) divided by transformants from (2), the enrichment ratio being a measure of the suitability of the DNA binding protein for displaying the peptide for specific binding to the receptor.

5. The method of claim 2, wherein the potential DNA binding proteins are variants of a natural DNA binding protein.

6. The method of claim 5, wherein the natural DNA binding protein is *lacI*.

7. The method of claim 6, wherein the vector lacks a *lacO* site.

8. The method of claim 7, wherein the potential DNA binding proteins are variants of a headpiece dimer comprising two *lac* headpieces joined by a linker.

9. The method of claim 2, further comprising contacting the complexes with bulk DNA to compete with the vectors for binding to the potential DNA binding proteins.

10. A method of constructing a random peptide library comprising:

(a) providing a recombinant DNA vector that encodes a DNA binding protein other than a phage coat protein;

(b) inserting into the coding sequence of the DNA binding protein a coding sequence for a random peptide such that the resulting vectors encode fusion proteins, each of which comprises the DNA binding protein and a random peptide;

(c) transforming host cells with the vectors; and

(d) culturing the transformed host cells under conditions suitable for expression of the fusion proteins, wherein the fusion proteins bind via the DNA binding protein to the vector with sufficient stability that complexes having a random peptide with a specific affinity for a receptor can be enriched by affinity purification on the receptor from complexes lacking a random peptide with a specific affinity for the receptor.

11. The method of claim 10, wherein the DNA binding protein is a nonsequence-specific DNA binding protein.

12. A method for screening a random peptide library for a peptide with specific affinity for a receptor, comprising:

(a) providing a peptide library wherein each member is a host cell transformed with a recombinant DNA vector that encodes a fusion protein comprising a DNA binding protein and a coding sequence for a random peptide, wherein members differ from other members with respect to the sequence of the random peptide, wherein the fusion proteins can bind via the DNA binding protein to the vector to form complexes having sufficient stability that complexes having a random peptide with a specific affinity for a receptor can be enriched by affinity purification to the receptor from complexes lacking a random peptide with a specific affinity for the receptor;

(b) lysing the cells transformed with the random peptide library under conditions such that the fusion protein remains bound to the vector that encodes the fusion protein;

(c) contacting the fusion proteins of the random peptide library with a receptor under conditions conducive to specific peptide-receptor binding; and

(d) isolating the vector that encodes a random peptide that binds to said receptor.

13. The method of claim 12, wherein the DNA binding protein has been isolated by the method of claim 1.

14. The method of claim 13, wherein the DNA binding protein is a nonsequence-specific DNA binding protein.

15. The method of claim 13, wherein the vector lacks a *lac*O site.

16. The method of claim 13, wherein the recombinant vector further comprises a DNA sequence with a specific affinity for the DNA binding protein.

17. The method of claim 12, wherein the host cells are bacteria.

18. The method of claim 17, wherein the bacteria are *E. coli*, and the vector is a plasmid.

19. The method of claim 18, wherein the DNA binding protein is a *lac* repressor protein comprising two *lac* headpieces joined by a first linker and the DNA binding protein is joined to the random peptide by a second linker.

20. The method of claim 19, wherein the first linker is GRCR, the two *lac* headpieces are designated A4.5 in Fig. 6 and the second linker is RSQE.

21. The method of claim 19, wherein the first linker is GRCR, the two *lac* headpieces are designated B4.5 in Fig. 6, and the second linker is GPNQ.

22. The method of claim 12, wherein the random peptide is located at the carboxy terminus of said fusion protein.

23. The method of claim 12, wherein the library has at least 10^6 different members.

24. The method of claim 12 further comprising:

(e) transforming a host cell with the vectors obtained in (d); and repeating (b), (c), and (d) with the host cells transformed in (e).

25. A recombinant DNA vector for constructing the random peptide library of claim 10, said vector comprising:

(a) a DNA sequence encoding the DNA binding protein;

(b) a promoter positioned so as to drive transcription of the DNA binding protein coding sequence;

(c) a coding sequence for a peptide inserted in the DNA binding protein coding sequence so that the coding sequences can be transcribed to produce an RNA transcript that can be translated to produce a fusion protein capable of binding to at least one DNA sequence in the vector.

26. A host cell transformed with the vector of claim 25.

27. A random peptide library comprising at least 10^6 different members, wherein each member is a host cell transformed with a recombinant DNA vector that encodes a fusion protein comprising a DNA binding protein other than a phage coat protein and a random peptide; and wherein members differ from other members with respect to the sequence of the random peptide, wherein the fusion proteins can bind via the DNA binding protein to the vector to form complexes having sufficient stability that complexes having a random peptide with a specific affinity for a receptor can be enriched by affinity purification to the receptor from complexes lacking a random peptide with a specific affinity for the receptor.